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Heavy Metal Uptake and Bioaccumulation by Mangrove Crab (*Goniopsis pelli*) from used Drilling Mud, Niger Delta, Nigeria

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ABSTRACT: This study was carried out to evaluate some heavy metal uptake and bioaccumulation in the meat, egg and shell of *G. pelli* from used drilling mud. The experimental approach involved the exposure of ten of the test organism *G. pelli* to six acute concentrations of drilling mud representing 0% (control), 10%, 20%, 30%, 40% and 50% for 96 hours in three replicates after the range finding test was conducted to determine the LC_{50} . From the LC_{50} , the test organism was further exposed to four sub-lethal concentrations of drilling mud (0%, 5%, 10% and 15%) for 21 days to evaluate the heavy metal uptake by the test organism. The result showed that heavy metal uptake increased in the order of meat ($<0.001-2.36\pm0.12$), shell ($<0.001-3.31\pm0.19$) and egg ($<0.001-0.54\pm0.05$) when compared with those in control tank. Copper was highest ($3.31\mu\text{g/g}$) in the shell and lowest ($0.26\mu\text{g/g}$) in the egg; zinc was highest ($2.36\mu\text{g/g}$) in the meat and lowest ($0.22\mu\text{g/g}$) in the shell; chromium was highest ($0.12\mu\text{g/g}$) in the meat and lowest ($0.06\mu\text{g/g}$) in the egg; cadmium was highest ($0.2\mu\text{g/g}$) in the shell and lowest ($0.001\mu\text{g/g}$) in the egg while lead had equal value (<0.001) in the meat, shell and egg of the test organism. This could pose a very serious health challenge to the consumers of *G. pelli* and other aquatic fishery resources if nothing is done to ensure the best practice in drilling activities in order to avoid pollution of the water bodies through drilling.

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Heavy metals constitute a very heterogeneous group of elements widely varied in their chemical properties and biological functions. They are kept under environmental pollutant category due to their toxic effects on plants, animals and human being. Bioaccumulation of heavy metals in living organisms defines the processes and pathways of pollutants from one trophic level to another. Heavy metal concentrations in the aquatic organism depict the past as well as the current pollution load in the environment in which the organism lives (Ravera *et al.*, 2003.). Pollution of the aquatic environment by inorganic chemicals has been considered a major threat to the aquatic organisms including fishes. Niger Delta is one of the world's most severely petroleum impacted ecosystems' undergoing damage from oil operations which is acute, chronic and cumulative. This has acted synergistically with other sources of environmental stress to result in a severely impaired coastal ecosystem and compromised the livelihoods and health of the inhabitants of the delta who rely on fisheries, subsistence agriculture and associated processing industries for their livelihood. Crabs are of economic importance to the people of Niger Delta. They are rich in protein and are used daily for food. Their nutritional value compares favourably with

those of domestic livestock and fish (Horsfall *et al.*, 2008). The objective of this study therefore, is to determine the lethal and sub-lethal effects of oil based drilling mud using the integration of bioaccumulation of heavy metals and oxidative stress analysis as measurement of stress indices to determine the susceptibility of this mangrove crab (*G. pelli*) in the environment.

MATERIALS AND METHOD

Study area: The samples were collected in Sivibilagbara, a protected mangrove swamp, and on four isolated, open, unvegetated intertidal flats along the Dor Nwezor channel of Bodo Creek ($4^{\circ}35'26.3''$ – $4^{\circ}36'29.7''$ N, $7^{\circ}15'30.2''$ – $7^{\circ}16'50.9''$ E) Figure 1 Sikoki and Zabbey (2006).

The samples were collected at different sampling stations, chosen to cover the various biotopes available on isolated intertidal flats fringing the Dor Nwezor main channel. The crabs were collected at low tide and transported to the Hydrobiology & Fisheries laboratory, Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt in a plastic bucket perforated to allow air.

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Fig.1: Map of Dor Nwezor Channel, Bodo Creek, Nigeria, indicating locations of sample sites Collection and Acclimatization

In the laboratory, the test organisms were immediately transferred to the holding tanks after length and weight measurements had been taken. They were conditioned for one week in the laboratory and left to acclimatize in eighteen plastic bowls of 30 litre capacity. This was to ensure that the test organisms will survive in the conditioned environment without showing signs of stress, damage or wounds from discolouration or unusual behaviour (Reish and Oshida, 1986).

Experimental Set-up: The test organisms were exposed to six different concentration tanks and the experiment conducted in three replicates for each concentration. In all 18 tanks were used for the species for the acute toxicity experiment.

Length and Weight Measurement: The length measurement of the crabs was determined using a meter rule on a measuring board in centimeters (cm3). An HX-Z electronic balance was used to measure the weight in grammes (g). Water was dabbed off crabs with filter paper before weighing Table 1.

Table 1: Size selection of the test organisms

Organism	Mean body length range	Mean weight range
<i>Goniopsis pelii</i>	13.5cm	29.83g

Preparation of Test Material for Acute Toxicity: The drilling mud obtained from Agip terminal brass used for the acute toxicity was prepared at the laboratory in advance and added immediately to the dilution water for the bioassay to obtain the different treatment concentrations. 100g/l in tank one, 200g/l, 300g/l, 400g/l and 500g/l in the various tanks and this was mixed using a mixer for about 30 minutes to ensure

homogeneity. This mixture was allowed to settle for one hour prior to the introduction of the test organisms (Reish and Oshida, 1986). The toxicant “drilling mud” was prepared using volumetric displacement in a 1 litre ratio of 1:9 (Finery, 1971). The following concentrations were obtained and used for the experiment 0% (control), 10%, 20%, 30%, 40% and 50% for the *Goniopsis pelii* (i.e. 10% OBM = 10ml/L) and a 1kg mud from where the test organisms were collected was added to each of the tanks.

Preparation of Bioassay Media for Acute Toxicity: A completely randomized design was carried out (Ogbeibu, 2005) in which healthy organisms after acclimatization were selected and placed in appropriate 50 litre tanks at 10 specimens per tank and covered with a net to allow for air and prevent the crabs from escaping.

Bioassay for Acute Toxicity: *Goniopsis pelii* (Mangrove crab) were exposed to OBM solution for 96 hours following the methods of Sprague (1970) and FAO (1973). Eighteen (18) plastic containers (30 litres) were used with six treatment concentrations of 0% (control), 10%, 20%, 30%, 40%, and 50%. The test was conducted under room temperature using static non-renewal bioassay (EPA, 2000). The exposure lasted for 96 hours (4 days) within which mortalities, general conditions and behaviours exhibited by the test organisms were observed and recorded at the following periods 15 minutes, 1 hour, 4 hours, 8 hours, then 12 hourly until the end of the experiment.

Determination of Heavy Metals in the Test Organism: Analysis of heavy metal content in the drilling mud used, meat of the exposed and unexposed organism, shell of the exposed and unexposed organism, eggs of the exposed and unexposed organisms, were carried out for chromium (Cr), cadmium (Cd), lead (Pb), copper (Cu) and zinc (Zn) at their various levels of metals present after exposure for 21 days. The well labeled samples were arranged in the oven and the charcoal heated. The heated charcoal was placed in the charcoal chamber of the local oven to dry the sample at a temperature between 76oC-85oC. The sample was placed in mortar after cleaning the mortar with hydrogen peroxide using cotton wool to avoid cross contamination of each sample and ground with the pestle to a powdered form. 10ml of hydrogen peroxide was measured using measuring cylinder and poured into a beaker containing 3g of the sample and boiled for 2-3 minutes to hasten the digestion. It was allowed to cool. Then 10ml of hydrochloric acid and 30ml of nitric acid were added and mixed homogenously. The beaker was placed on the heat source and allowed to

heat until it got digested at a temperature of 65oC-70oC

RESULTS AND DISCUSSION

Physicochemical Parameters: The summarized data on the physicochemical parameters showing the mean values and the standard error obtained are presented in Fig.2. The temperature values had a narrow range throughout the study with the minimum mean temperature of 26.73oC recorded at treatment exposed to 40% OBM and the maximum mean temperature of 29.63oC recorded for treatment at 0% (control). There was significant difference $p \geq 0.05$ in temperature for the concentration used and the species exposed. A narrow range was obtained for pH with mean values that ranged from 6.33-6.83 with significant difference of $p \geq 0.05$ between the concentrations. Dissolved oxygen (DO) values obtained ranged from 4.20mg/l-5.80mg/l. It tended to vary slightly from one concentration to another with decrease from the lowest concentration to the highest concentration. However, there was no significant difference in the various concentrations. Total Dissolved Solid (TDS) values tend to vary slightly from 14.20 to 15.60 as the concentration increased from 0% -50%. There was no significant difference that existed between the various concentrations. Similarly, the value for conductivity decreased from 19.57 to 17.56 with increased concentrations. The results from the study showed that the water quality fell within the range recommended and the variations that occurred among the parameters measured were within the tolerable ranges throughout

the bioassay tests (Bolarin and Halton, 2009). The dissolved oxygen of 4.2mg/l and 5.8mg/l obtained in the study was normal compared to stipulated limits and is conducive for the survival of the test organism (*Goniopsis pelii*) under non-renewable static bioassay. This result agrees with the work of Okogbue (2014) who reported that dissolved oxygen ranged from 3.45mg/l to 5.95ml/l for *M. macrobrachion* exposed to N.P.K. fertilizer as toxicant was conducive for the survival of the test organism. Similarly, the temperature range of 26oC to 29oC fell within the acceptable limit (EIFAC, 2003). This result agrees with the results reported by Gbadebo *et al.*, (2010) and Hartley *et al.*, (2003). The pH values of 6.3 – 6.8 obtained from this study conforms to the recommended range of 6 – 9 for the survival of tropical fishes. The high salinity range of 15.0 – 15.3 obtained from the study could be attributed to the saline nature of brackish waters. The values for total dissolved solid (TDS) was found to be lowest (14.20) in the control tank and highest (15.60) in 40% concentration tank. The increase in TDS values could be attributed to increase in the concentration of the toxicant and this result agrees with the result of other authors (Okogbue; 2014, Nwakanma, 2012). Similarly, the values for conductivity varied slightly as the concentration increased. The variations in the water quality observed did not adversely alter the water quality integrity and were in conformity with the finding of different authors (Wade *et al.*, 2002; Omoregie *et al.*, 1990; Annue and Ajike, 1999; Aguiwo, 2002; Okoli-Annuobi *et al.*, 2002).

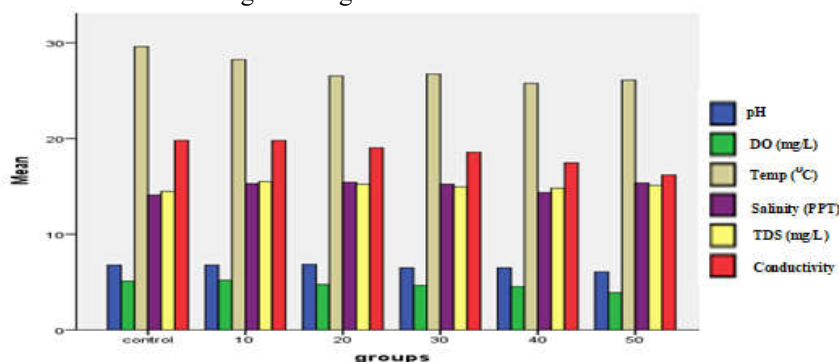


Fig.2: Mean values of physicochemical parameters of the test media against the concentrations

Mortality: The effect of the different concentrations of OBM on *Goniopsis pelii* showed marked variations during the course of the experiment. No mortality was recorded in the control test media Table 2. However, mortality increased with increase in drilling mud concentrations over time. After 24hours, only one specimen died in the 100g/l and 200g/l concentration respectively; while mortality increased to 4 in 300g/l, 8 in 400g/l and 13 in 500g/l concentrations respectively. However, after 96hours of exposure, a

total of 3 representing 9.99% died in the 100g/l concentration, 15 in 200g/l, 17 in 300g/l, 25 in 400g/l and 30 in 500g/l representing 49.99%, 56.65%, 83.31% and 99.99% mortality Table 2. The result of the analysis showed that as the concentration increased, more death were recorded and as the time increased, the LC₅₀ (200g/l conc.) reduced. Generally, mortality is indicative of the end point for toxicity test (Odiete, 2009). Therefore, the main biological parameter monitored in this study among others is

mortality. From the study, mortality increased with increase in concentration of drilling mud over time of exposure which was in agreement with previous findings by other authors (OGP, 2003, Bowmer *et al.*, 1996) at 24 hours, mortalities of 0% was recorded for the control tank, 3.33% for 100g/l, 3.33% for 200g/l, 13.33% for 300g/l, 26.66% for 400g/l and 43.33% for 500g/l concentrations respectively. However the mortality increased with time and was found to have risen to 9.99% in 100g/l, 49.99% in 200g/l, 56.65% 300g/l, 83.31% in 400g/l and 99.99% in 500g/l concentrations respectively after 96hours of exposure. However, Nwakanma (2012) reported mortalities of 0%, 3%, 10%, 13% and 20% after 24hours of exposure to *P. papilio* to toxicant and 30%, 40%, 50%, 57% and

73% at 96hours. Although the values varied slightly from the result of this study but the result shows that there is an increase in mortality as the concentration increases with time. Similarly, Nte (2014), Vincent-Akpu (2006), Nafagha (2014) and Okogbue (2014) also noted that the mortality rate increased with increase in concentration as the time increased in their various reports. The absence of mortality in the control tank shows that mortality was caused by the drilling mud. This confirms that drilling muds are toxic to the test organism (*Goniopsis pelii*). Neff *et al.*, (1981) also noted that if mud aqueous fraction was renewed daily, its toxicity will decrease seven fold, demonstrating that the toxic component may be lost from solution by volatilization.

Table 2: Mortality (mean numbers and percentage) of *Goniopsis pelii* exposed to OBM for 96 hours

Time	24 hours		48 hours		72 hours		96 hours	
Conc. (%)	No. of organism	Rep	Mortality	% mortality	Mortality	% mortality	Mortality	% mortality
Control	10	3	-	-	-	-	-	-
10	10	3	1	3.33	1	3.33	0	0
20	10	3	1	3.33	2	6.66	6	20
30	10	3	4	13.33	2	6.66	4	13.33
40	10	3	8	26.66	5	16.66	1	3.33
50	10	3	13	43.33	7	23.33	3	10

Uptake of Heavy Metal: The results of the uptake of heavy metals in the shell, meat and egg of the test organism exposed to toxicant and those unexposed to toxicant are presented in the Figures 3-6.

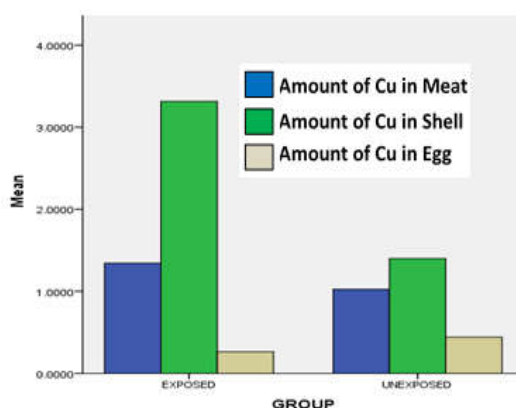


Fig 3: Relationship between the copper concentration in the meat, shell and egg of test organisms exposed to drilling mud and those unexposed after 21 days of sub-lethal bioassay.

The values for each metal were as follows: Copper 1.013 $\mu\text{g/g}$, 3.32 $\mu\text{g/g}$ and 0.2/g in meat, shell and egg respectively of the test organism exposed to drilling fluid and 1.025 $\mu\text{g/g}$, 1.4 $\mu\text{g/g}$ and 0.447 $\mu\text{g/g}$ in the control organism respectively Fig. 3.

Chromium 0.12 $\mu\text{g/g}$, 0.098 $\mu\text{g/g}$ and 0.067 $\mu\text{g/g}$ in the meat, shell and egg of the test organism exposed to drilling fluid respectively and 0.105 $\mu\text{g/g}$, 0.083 $\mu\text{g/g}$

and 0.0218 $\mu\text{g/g}$ for the meat, shell and egg of those not exposed to drilling fluid Fig. 4.

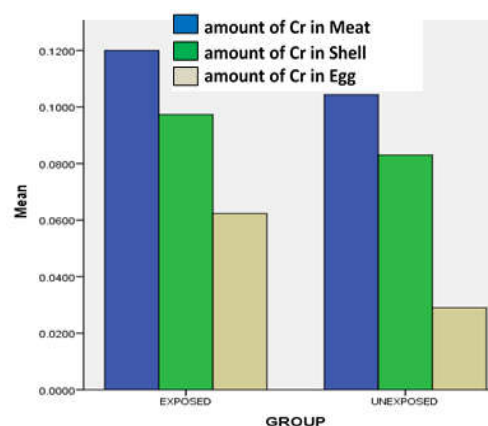


Fig.4: Relationship between the chromium concentration in the meat, shell and egg of test organisms exposed to drilling mud and those unexposed after 21 days of sub-lethal bioassay

For cadmium it was 0.002 $\mu\text{g/g}$, 0.021 $\mu\text{g/g}$, and <0.001 $\mu\text{g/g}$ for meat, shell and egg of test organism exposed to drilling mud and <0.001 $\mu\text{g/g}$, <0.001 $\mu\text{g/g}$ and 0.003 $\mu\text{g/g}$ for the meat, shell and egg of the test organism not exposed to toxicant respectively Fig. 5. For zinc it was 1.213 $\mu\text{g/g}$, 0.211 $\mu\text{g/g}$, and 0.51 $\mu\text{g/g}$ for meat, shell and egg of test organisms exposed to the toxicant and 0.99 $\mu\text{g/g}$, 0.55 $\mu\text{g/g}$ and 0.912 $\mu\text{g/g}$ for the meat, shell and egg of the test organism in the control Fig. 6.

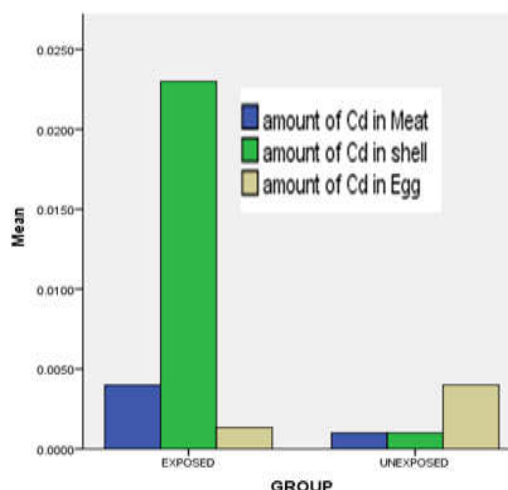


Fig. 5: Relationship between the Cadmium concentration in the meat, shell and egg of test organisms exposed to drilling mud and those unexposed after 21 days of sub-lethal bioassay

Lead was found to have the same value of $<0.001\mu\text{g/g}$ in meat, shell and egg of the test organism exposed and unexposed to toxicant. In summary, Copper (Cu) was found to be highest in the shell of the test organism exposed to drilling mud, zinc was highest in the meat and egg while lead was found to be lowest in all. Statistical analysis showed no significant difference in lead (Pb) Chromium (Cr) and Cadmium (Cd) for test organisms exposed to the toxicant Fig. 7.

Crabs may be affected directly by the uptake of oil through water contaminated sediments and food

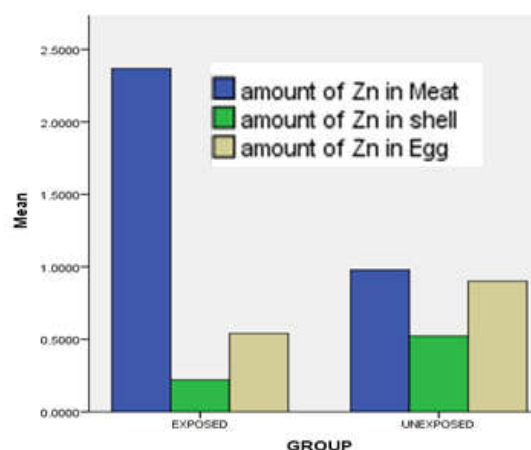


Fig. 6: Relationship between the zinc concentration in the meat, shell and egg of test organisms exposed to drilling mud and those unexposed after 21 days of sub-lethal bioassay

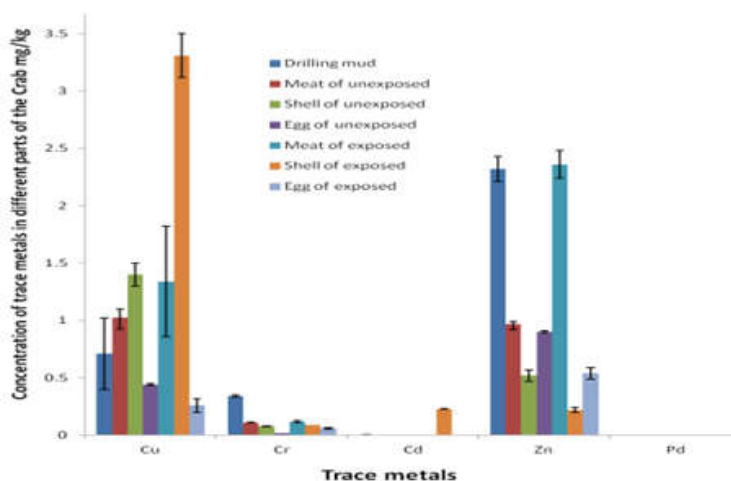


Fig. 7: Summary of heavy metal uptake in the meat, egg and shell of the test organism exposed to toxicant and those not exposed

Copper had the highest concentration in the shell of the test organism followed by the meat and then the egg in both the exposed and unexposed. This may be as a result of the direct contact of the shell to the toxicant for a longer period of time than the other parts. The intake of chromium was found to be highest

in the meat of the exposed when compared with the unexposed. Cadmium was found to be highest in the shell of the exposed when compared with the meat and the egg. Zinc intake was found to be highest in the meat of the exposed when compared with the shell and

the egg. Lead had same value for all compartments in both the exposed to drilling mud and unexposed.

Conclusion: From the results obtained in this study, it has been established that drilling mud has toxic effect on crabs which can destroy their role in the ecosystem thereby causing congestion in the ecosystem. There could be a multiple effect on human health when these contaminated crabs are consumed. Therefore, this study has added to scientific knowledge by providing information on the consequences of the toxic effect of used drilling mud on a *G.pelli* and potentially on human health since the tested heavy metals could bioaccumulate in the edible tissues of the crab.

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